



The Egyptian German Society for Zoology
The Journal of Basic & Applied Zoology

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Lipid profile and levels of homocysteine and total antioxidant capacity in plasma of rats with experimental thyroid disorders



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Received 23 September 2013; revised 28 September 2014; accepted 1 January 2015

Available online 31 January 2015

KEYWORDS

Homocysteine;
 Hypothyroidism;
 Hyperthyroidism;
 Lipid profile;
 Total antioxidant capacity;
 Rats

Abstract This study focuses on the relationship between serum levels of cholesterol and homocysteine with that of total antioxidant capacity in rats with thyroid dysfunction.

Adult male Wistar rats were divided into three groups, a control (euthyroid), hypothyroidism and hyperthyroidism, each of them containing ten rats. Hypothyroidism was induced by administration of 0.1% aminotriazole in drinking water for 3 weeks. Hyperthyroidism was induced by chronic subcutaneous injection of L-thyroxine (100 µg/day, dissolved in 200 µL saline solution/100 g body weight) for 3 weeks. The control and hypothyroid groups were injected subcutaneously with the same volume of saline solution.

Results showed that hyperthyroidism is characterized by reduced serum thyroid stimulating hormone (TSH) levels despite increased free thyroxine (FT₄) and free triiodothyronine (FT₃) levels.

Significant ($p < 0.05$) elevation in serum levels of total homocysteine (t-Hcy) is reflected by a decrease in serum total antioxidant capacity (TAC) production in hypothyroidism comparing to control.

There was a significant ($p < 0.05$) elevation in serum levels of lipid profile (cholesterol, triglyceride and LDL) in hypothyroidism. Significant ($p < 0.05$) reduction occurred in the levels of cholesterol and triglyceride in hyperthyroidism. The association of hyperhomocysteinemia and lipid abnormalities occurring in hypothyroidism may represent a dynamic atherogenic state.

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Introduction

Thyroid disease, namely hypothyroidism and hyperthyroidism, constitutes the most common endocrine abnormality in

recent years, diagnosed either in subclinical or clinical form. Thyroid disease is associated with various metabolic abnormalities, due to the effects of thyroid hormones on nearly all major metabolic pathways.

Thyroid hormones, thyroxine (T₄), and triiodothyronine (T₃) play an important role in all major metabolic pathways. They regulate the basal energy expenditure through their effect

Peer review under responsibility of The Egyptian German Society for Zoology.

<http://dx.doi.org/10.1016/j.jobaz.2015.01.001>

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on protein, carbohydrate, and lipid metabolism. This might be a direct effect or an indirect effect by modification of other regulatory hormones such as insulin or catecholamines (Kim, 2008). Many studies reported that thyroid hormones stimulate cholesterol synthesis by inducing 3-hydroxy-3-methyl-glutaryl coenzyme A reductase in the liver (Cachefo et al., 2001; Beylot, 2001). In addition thyroid hormones influence all aspects of lipid metabolism including synthesis, mobilization, and degradation. Furthermore, thyroid hormones affect lipoprotein lipase activity and thus, the hydrolysis of triglycerides into very-low density lipoprotein (VLDL) and chylomicrons into fatty acids and glycerol (Cachefo et al., 2001). Finally, thyroid hormones modulate lipid metabolism by upregulation of the low density lipoprotein (LDL) receptors, which results in enhanced catabolism of the LDL particles.

In hypothyroidism, lipoprotein lipase activity in the adipose tissue has been found normal or decreased, in addition to decreased hepatic lipase activity resulting in normal or high levels of triglycerides (Abrams et al., 1981). In hyperthyroidism, although lipoprotein lipase activity is usually normal (Tan et al., 1998), an increased liver fatty acid synthesis and oxidation are observed due to enhanced acetyl-CoA carboxylase 1 and carnitine palmitoyltransferase Ia expression leading to increased VLDL biosynthesis (Liu and Brent, 2010). Moreover, hyperthyroidism is characterized by reduced serum TSH levels despite increased free thyroxine (FT₄) and free triiodothyronine (FT₃) levels.

Dyslipidemia is a common metabolic abnormality in patients with thyroid disease, either in the overt or subclinical forms of the disease, and constitutes the end result of the effect of thyroid hormones in all aspects of lipid metabolism leading to various quantitative and/or qualitative changes of triglycerides, phospholipids, cholesterol, and other lipoproteins. Dyslipidemia also occurs due to the coexisting metabolic abnormalities in thyroid disease including oxidative stress and insulin resistance, which induce further or aggravate the existing dyslipidemia, via a vice-vicious cycle (Santi et al., 2010; Tagami et al., 2010).

Moreover, homocysteine is a marker for low thyroid and low B vitamins (should be less than 9) so the higher total homocysteine concentrations seen in the elderly may be caused by many factors including malabsorption of B12 or a suboptimal intake of B-vitamins (especially vitamin B₁₂), reduced kidney function, medications that reduce the absorption of vitamins (as in the case of H₂ receptor antagonists or proton-pump inhibitors reducing B12 absorption) (Ruscin et al., 2002) or increase in the catabolism of the vitamins (as in the case of metformin reducing blood levels of B12 and folic acid). Certain diseases are associated with higher homocysteine levels, as can such lifestyle factors as smoking (Targher et al., 2000), coffee consumption (Temple et al., 2000), and excessive alcohol intake (Sakuta and Suzuki, 2005). Lack of exercise, obesity (Yun et al., 2013) and stress are also associated with hyperhomocysteinemia.

Thus, the present study was carried out to investigate the changes in lipid profile associated with disturbances in serum levels of cholesterol and homocysteine with that of total antioxidant capacity in rats as a result of experimentally-induced hypo- and hyperthyroidism.

Material and methods

Thirty adult male Wistar rats (200–250 g) were used for the current study after being procured from the Animal House of El-Nile Company for Pharmaceutical Products, Cairo, Egypt. The animals were acclimatized for 2 weeks in the Animal House of Zoology Department, Women's College, Ain Shams University before induction of hypothyroidism or hyperthyroidism in them. Five rats were housed per wire floored cage in an air-conditioned room ($22 \pm 2^\circ\text{C}$) with 12 h light/dark cycle and had free access to standard laboratory chow diet (El Nasr Co., Cairo, Egypt) according to National Research Council (NRC, 1977) and water *ad libitum*. The protocol of this study was approved by the Department of Zoology Council, Women's College, Ain Shams University, Egypt, which has an ethical authority.

Animals were divided into three groups, control animal (euthyroid) group, hypothyroidism rat group and hyperthyroidism rat group, each of them containing ten rats. Hypothyroidism was induced by administration of 0.1% aminotriazole (Sigma Chem. Co., St. Louis, MO, USA) in drinking water for 3 weeks as previously described by Lopez et al. (2001). Whereas, hyperthyroidism was induced by chronic subcutaneous injection of L-thyroxine (100 µg/day, dissolved in 200 µL saline solution/100 g body weight) (Sigma Chem. Co., St. Louis, MO, USA) for 3 weeks according to Lopez et al. (2002). The control animal (euthyroid) group and hypothyroid animals were injected subcutaneously with the same volume of saline solution (0.9% NaCl). At the end of each treatment, animals were dissected under slight anesthesia by ether, blood samples were collected by heart puncture, centrifuged and the sera were separated and stored at -20°C until assayed.

Estimation of serum thyroid stimulating hormone (TSH)

Thyroid stimulating hormone (TSH) was assayed by radioimmunoassay (RIA) kit using the solid phase component system (Phoenix Pharmaceuticals, Inc., USA) as described by Patrono and Peskar (1987).

Estimation of serum hormonal profile

Serum total triiodothyronine (T₃) and total thyroxine (T₄) levels were estimated by a radioimmunoassay method kit using solid phase component system according to Ekins (1978) and Chopra et al. (1972). The kits were purchased from Diagnostic Product corporation (DPC), USA. Serum free triiodothyronine (FT₃) and free thyroxine (FT₄) levels were estimated by a radioimmunoassay method kit using solid phase component system according to Ekins (1978) and Siegel et al. (1978). The kits were purchased from Phoenix Pharmaceuticals, Inc., USA.

Estimation of serum T₃-uptake

T₃-uptake was assayed using ELISA techniques. The kits were purchased from Immuno-Biological Laboratories, Inc. (IBL-America), Minneapolis, USA according to Witherspoon et al. (1981).

Determination of homocysteine level

The levels of total homocysteine (t-Hcy) were assayed by the aid of ELISA (Sandwich immunoassay technique) using commercial kit (CUSABIO, China) according to [Primus et al. \(1988\)](#).

Estimation of serum lipid profile

Serum total cholesterol, triglycerides and HDL-cholesterol were estimated enzymatically according to [Watson \(1960\)](#), [Fossati et al. \(1982\)](#), and [Freidewald et al. \(1972\)](#), respectively using commercial kits from Ran-dox, Ltd., Co. (UK). LDL-cholesterol was calculated according to the equation of [Assmann et al. \(1984\)](#) as follows:

$$\text{LDL} - \text{Chol.} = \text{Total Chol.} - [\text{TG}/5 - \text{HDL} - \text{Chol.}]$$

Determination of total antioxidant capacity (TAC) concentration

Total antioxidant capacity (TAC) was measured according to the method of [Koracevic et al. \(2001\)](#), using EIA kit that was purchased from Labor Diagnostika Nord Co., Germany.

Statistical analysis

Statistical deference between the means was assayed by using analysis of variance (ANOVA) followed by Duncan's multiple range tests according to [Snedecor and Cochran \(1982\)](#).

Result and discussion

Thyroid gland function regulates a wide range of metabolic events, it significantly affects lipoprotein metabolism and as a result cardio vascular disease (CVD) risk ([Duntas, 2002](#)). Thyroid hormones affect cholesteryl ester transfer protein and hepatic lipase activity, which are increased in hyperthyroidism and decreased in hypothyroidism, with consequent changes not only in total high-density lipoprotein (HDL) but also in HDL subfraction levels ([Berti et al., 2001](#)). Thyroid disorders are known to influence lipid metabolism and are common in dyslipidemic patients. Overt and subclinical hypothyroidism have an adverse effect on the serum lipid profile that may predispose to the development of atherosclerotic

disease. In addition, levels of total and LDL cholesterol tend to increase as the thyroid function declines. Therefore, hypothyroidism constitutes a significant cause of secondary dyslipidemia. In addition, thyroid failure is accompanied by an increase in plasma homocysteine levels with its known adverse effect on the cardiovascular system ([Biciková et al., 2003](#)).

Homocysteine a type of amino acid that is naturally found in blood plasma is not harmful at normal levels, but when its levels are too high, health problems can result. If unhealthy levels of homocysteine accumulate in the blood the delicate lining of an artery (endothelium) can be damaged. Also, homocysteine can both initiate and potentiate atherosclerosis. For example, homocysteine-induced injury to the arterial wall is one of the factors that can initiate the process of atherosclerosis, leading to endothelial dysfunction and eventually to heart attacks and strokes ([Gallai et al., 2001](#); [Papatheodorou and Weiss, 2007](#)). Several studies have shown that homocysteine can inflict damage to the arterial wall via multiple destructive molecular mechanisms ([Hofmann et al., 2001](#); [Zeng et al., 2003](#); [Osanai et al., 2010](#)).

In the present study, rat was used as an animal model for induction of hypothyroidism and hyperthyroidism. The levels of total homocysteine, total antioxidant capacity and correlation with the levels of cholesterol and other measured parameters were evaluated in thyroid dysfunction.

[Table 1](#) illustrates the changes in thyroid profile levels in hypo- and hyperthyroidism rats. In hypothyroidism rat group there was a high significant ($p < 0.05$) elevation in serum levels of thyroid stimulating hormone (TSH) while there was a significant ($p < 0.05$) decrease in serum levels of total thyroxine (TT₄), total triiodothyronine (TT₃), free T₄ (FT₄), free T₃ (FT₃) and T₃-uptake. In relation to the euthyroid rat group, a significant ($p < 0.05$) decrease in the serum levels of TSH was observed in the hyperthyroidism rat group. Moreover, a significant ($p < 0.05$) increase in serum levels of TT₄, TT₃, FT₄, FT₃ and T₃-uptake was noted.

The present results suggest that the changes in thyroid profile levels in both hypo- and hyperthyroidism rat group may be due to the disturbance in the hypothalamic-pituitary-thyroid axis, the increment in the conversion of T₃ to T₄ or/and the elevation in the formation of reverse T₃ (rT₃) concentration. Furthermore, there was a relationship between the concentrations of thyroid hormones (T₃ and T₄) in serum and hyperlipidemia which is evident and the thyroid activity has an adverse effect on all plasma lipids ([Deuel, 1955](#)). Thyroid

Table 1 Changes in thyroid profile levels in hypo- and hyperthyroidism rats.

Parameters	Euthyroid rat group	Hypothyroidism rat group	Hyperthyroidism rat group
TSH (mIU/mL)	1.19 ± 0.016 ^a	3.40 ± 0.106 ^b	0.41 ± 0.006 ^c
T ₄ (µg/mL)	3.72 ± 0.065 ^a	1.63 ± 0.037 ^b	8.11 ± 0.118 ^c
T ₃ (ng/mL)	62.12 ± 1.12 ^a	36.53 ± 0.79 ^b	148.82 ± 3.47 ^c
FT ₄ (ng/mL)	0.38 ± 0.005 ^a	0.17 ± 0.003 ^b	1.02 ± 0.011 ^c
FT ₃ (pg/mL)	0.71 ± 0.009 ^a	0.46 ± 0.005 ^b	2.43 ± 0.028 ^c
T ₃ -uptake (%)	10.97 ± 0.42 ^a	5.89 ± 0.23 ^b	19.06 ± 0.81 ^c

Values are expressed as means ± S.E, $n = 10$.

^a Significant at $p < 0.05$ between the groups in the same rows.

^b Significant at $p < 0.05$ between the groups in the same rows.

^c Significant at $p < 0.05$ between the groups in the same rows.

Table 2 Changes in homocysteine, lipid profile and total antioxidant capacity levels in hypo- and hyperthyroidism rats.

Parameters	Euthyroid rat group	Hypothyroidism rat group	Hyperthyroidism rat group
Total Hcy ($\mu\text{mol/L}$)	10.02 \pm 0.69 ^a	12.67 \pm 0.84 ^b	9.94 \pm 0.66 ^a
Cholesterol (mg/mL)	56.72 \pm 1.11 ^a	90.35 \pm 1.66 ^b	49.21 \pm 1.19 ^c
Triglycerides (mg/mL)	69.19 \pm 1.32 ^a	102.47 \pm 2.11 ^b	48.93 \pm 1.48 ^c
HDL (mg/mL)	15.12 \pm 0.63 ^a	15.18 \pm 0.65 ^a	11.45 \pm 0.51 ^b
LDL (mg/mL)	27.76 \pm 0.81 ^a	54.68 \pm 1.32 ^b	27.97 \pm 0.86 ^a
TAC (mmol/L)	1.437 \pm 0.045 ^a	0.978 \pm 0.029 ^b	1.429 \pm 0.031 ^a

Values are expressed as means \pm S.E, $n = 10$.

^a Significant at $p < 0.05$ between the groups in the same rows ($\mu\text{mol/L}$).

^b Significant at $p < 0.05$ between the groups in the same rows ($\mu\text{mol/L}$).

^c Significant at $p < 0.05$ between the groups in the same rows ($\mu\text{mol/L}$).

hormones, especially triiodothyronine (T_3), induce low-density lipoprotein (LDL) receptor gene expression in the liver, enhancing LDL clearance and explaining the decreased or increased LDL levels that were observed in hyperthyroidism and hypothyroidism, respectively.

A significant elevation in serum levels of total homocysteine (Hcy), total cholesterol triglycerides and LDL was observed in relation to the euthyroid rat group whereas a significant ($p < 0.05$) depletion occurred in the total antioxidant capacity (TAC) in the hypothyroidism rat group but no marked changes were observed in serum levels of HDL-C. In the hyperthyroidism rat group, the changes in serum levels of total homocysteine were not significant. Further, a notable decrease in total cholesterol, triglycerides and HDL was also observed. In addition, no remarkable changes in serum levels of LDL-C and TAC were noticed (Table 2). These results may be attributed to the disturbances in the fat absorption and its metabolism, alteration in de Novo lipogenesis, changes in the β -oxidation of fatty acids in the matrix of mitochondria and disturbances in the hypothalamus–pituitary–thyroid axis (HPTA). The obtained data in the current investigation were in agreement with several studies that reported an overt hypothyroidism is characterized by hypercholesterolemia and a marked increase in low-density lipoproteins (LDL) and apolipoprotein B (Duntas, 2002). These changes accelerate atherosclerosis, which causes coronary artery disease (Kritz-Silverstein et al., 2009; Rizos et al., 2011). Also, high homocysteine levels in the blood cause cholesterol to change to oxidized low-density lipoprotein, which damages the arteries by creation of plaque inside artery walls (McCully, 1996). Some forms of homocysteine have been shown to damage the inner walls of blood vessels directly (Jakubowski, 2003). Another important finding was the significant correlation between serum levels of homocysteine, total cholesterol and LDL-C with total antioxidant capacity was noticed in the hypothyroidism rat group but the correlation was not significant in the case of hyperthyroidism rat group.

Altered lipid profile is a well-known manifestation of thyroid dysfunction. Both plasma LDL-C and HDL-C increase in hypothyroidism and decrease in hyperthyroidism (Diekmann et al., 2000). These changes in the lipid profile are explained by the regulatory effect of thyroid hormones on the activity of some key enzymes of lipoprotein metabolism. Specifically, the thyroid hormone stimulates the hepatic de novo cholesterol synthesis by inducing the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase that catalyzes

the conversion of HMG-CoA to mevalonate, the first step in the biosynthesis of cholesterol. This results in an enhanced intracellular cholesterol concentration in hyperthyroidism and a decreased one in hypothyroidism (Duntas, 2002).

Most of the existing studies support lower total and LDL cholesterol levels in patients with hyperthyroidism while only a few data support no change (Yavuz et al., 2004). Lower triglycerides, HDL, apoA1, apoB, and Lp(a) levels have been found in patients with hyperthyroidism compared with euthyroid controls (Alterihy et al., 2012).

Thyroid disease is related to the development of dyslipidemia which is a well-known atherogenic factor. Further, dyslipidemia induces insulin resistance oxidative stress, via a vice–vicious cycle (Santi et al., 2010). Insulin resistance, hypertension, inflammation, oxidative stress, and coagulation deficits are also promoted by thyroid disease, independently of dyslipidemia (Biondi and Klein, 2004). The above associations support a multifactorial origin of atherosclerosis in thyroid disease, with dyslipidemia playing an important role (Fazio et al., 2004).

The risk of heart disease increases proportionally with increasing TSH, even in subclinical hypothyroidism (Rodondi et al., 2010). Hypothyroidism that is caused by autoimmune reactions is associated with stiffening of the blood vessels (Stamatelopoulos et al., 2009). Thyroid hormone replacement may slow the progression of coronary heart disease by inhibiting the progression of plaques (Caparevic et al., 2003). Moreover, treating hypothyroid patients with thyroid hormone replacement might attenuate homocysteine levels, an independent risk factor for cardiovascular disease. A strong inverse relationship between homocysteine and free thyroid hormones confirms the effect of thyroid hormones on homocysteine metabolism (Biciková et al., 2003). Recent investigations in humans have shown that in case of high levels of thyroid stimulating hormone (TSH) values, there is a linear increase in total cholesterol, LDL-C, and TGs in addition to a linear decrease in HDL-C (Asvold et al., 2007; Alterihy et al., 2012).

Hyperhomocysteinemia, a newly emerged independent risk factor coronary artery diseases, is one of the main factors that cause various diseases, such as cancer, (Poirier et al., 2001) atherosclerosis (Merkel, 2004) diabetes, (Luis et al., 2004) and some other aged-related illnesses including Alzheimer's disease, (Nilsson et al., 2002). Homocysteine is formed by demethylation of essential amino acid methionine. Thus, an imbalance in dietary methionine may contribute to the development of atherosclerosis by increasing homocysteine levels.

Kapoor et al. (2008), demonstrated an increase in homocysteine, total cholesterol, triglycerides, LDL and TBARS levels in myocardial homogenates in methionine-treated rats with a notable decrease in HDL levels was also seen. Furthermore, high cholesterol has been the focus of the medical community as the cause of heart attacks and strokes. Some factors such as: urban residence, butter consumption, hypertension and intellectual work may contribute to the etiology of atherosclerosis through their impact on serum lipid fractions (Mohamed et al., 2008).

Oxidative stress induced by homocysteine is reflected by a decrease in serum total anti-oxidant capacity. The oxidative stress resulting from elevated serum Hcy can oxidize membrane lipids and proteins and stimulate the activation of NF- κ B, and consequently increase the expression of inflammatory factors in vivo. Hcy can be converted to a highly reactive thiolactone which is able to react with proteins forming-NH-CO-adducts, thus affecting body proteins and enzymes (Ramakrishnan et al., 2006). Such an effect may contribute to atherogenesis by enhancing the inflammatory response of the vascular endothelium (Zhang and Mild, 2004). Messarah et al. (2007) explain the thyroid activity variation in relation to the lipid peroxidation and the tissue contents of the enzymatic and the non-enzymatic antioxidants and show the occurrence of a state of oxidizing stress in relation to hyperthyroidism. Homocysteine like sulfhydryl compound can promote the oxidation of LDL, reduce the concentration of HDL cholesterol in plasma by inhibiting the synthesis of apo A-I, the main HDL apolipoprotein and increase the serum levels of MDA (Barter and Rye, 2006). Hcy induced lipid dysregulation is an important mechanism linking Hcy to the development of atherosclerosis. Thyroid hormones are physiologic modulators of both tissue oxidative stress and protein degradation. The mechanism linking hypothyroidism with oxidative stress is unknown. Rahbani-Nobar et al. (2004) demonstrated the correlation between serum levels of homocysteine, total cholesterol and LDL with total antioxidant capacity in hypothyroidism and suggests that there is an over production of free radicals in these patients that is contributed to abnormalities seen in homocysteine and cholesterol metabolism.

It is a well-established fact that thyroid hormones regulate the expression of enzymes involved in all steps of lipid metabolism leading to the development of qualitative and quantitative changes of lipids, in thyroid disease. Dyslipidemia coexists with other metabolic abnormalities, including, hypertension, insulin resistance, and oxidative stress, all of them being risk factors for cardiovascular disease. Also, elevation of total plasma concentration of homocysteine (t-Hcy) is an important and independent risk factor for cardiovascular disease. Additionally, hypothyroidism is possibly also associated with an increased risk for coronary artery disease, which may be related to atherogenic changes in the lipid profile (Alterihy et al., 2012; Yun et al., 2013).

In conclusion, a strong relationship between serum homocysteine levels and lipid profile concentrations especially the concentrations of cholesterol in hypothyroidism may increase cardiovascular risk (CVR). So, determination of serum levels of thyroid profile is recommended in subjects with unexplained hyperhomocysteinemia and hypercholesterolemia.

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